SPEED RIVER NITRIFICATION STUDY

Microbiological Survey Report

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MINISTRY OF THE ENVIRONMENT

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SUMMARY

The population distributions of nitrifying bacteria were examined in Speed River downstream of Guelph Sewage Treatment Plant (STP). Both ammonia-oxidizers (Nitrosomonas) and nitrite-oxidizers (Nitrobacter) were found in substantial numbers in waters and sediments. The strikingly high densities of these bacteria in sediments indicate that the river bed may be the site of nitrification.

Maximum levels of Nitrosomonas and Nitrobacter were observed both in waters and sediments downstream of STP between stations S6 and S10. This stretch of the Speed river may be classified as the "Zone of Nitrification".

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INTRODUCTION

Nitrification is the process of oxidation of organic nitrogen and free ammonia nitrogen to nitrite (partial oxidation) and nitrate (complete oxidation). In nature this process is carried out by a small group of autotrophic bacteria of the genera Nitrosomonas and Nitrobacter. The first oxidation step ($NH_4^+ \dashrightarrow NO_2^-$) is accomplished by members of Nitrosomonas, whereas the second oxidation step ($NO_2^- \longrightarrow NO_3^-$) is mediated by members of Nitrobacter.

Little of the substantial amounts of ammonia present in sewage effluent is oxidized during conventional biological treatment. Nitrification of the effluent ammonia in receiving waters by nitrifying bacteria can consume significant amounts (3.22 mg O_2/mg NH_4^+-N and 1.11 mg O_2/ mg NO_2^--N) (15) of dissolved oxygen resulting in oxygen deficiencies. This has been well documented (1, 2, 3) and also reported in recent studies in the Thames River and Grand River Basins (4, 5).

An investigation was designed to examine in detail the nature of nitrification and total oxygen demand of effluents under variable environmental conditions in the Speed River downstream of the Guelph Sewage Treatment Plant (STP). As part of this study, a two-day microbiological survey was carried out to determine the distribution and concentration of nitrifying bacteria in water and sediments. This survey was requested by the Water Modelling Section, Water Resources Branch, Ministry of the Environment.

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MATERIALS AND METHODS

Sample Collection and Stations

On September 8 and 9, 1976, surface water samples were collected in presterilized glass sampling bottles (ca. 170 ml) from six stations in the Speed River (Figure 1). Bottom sediments were collected in presterilized Nalgene wide mouth jars (ca. 125 ml) only on the first day of the survey. All samples were transported on ice to the laboratory and were analyzed within 24 hours of collection.

Microbiological Determinations

Nitrifying Bacteria

Autotrophic nitrifying bacteria were enumerated both in water and sediment by a most probable number (MPN) procedure using 10-fold dilutions with three tubes per dilution. The broth media used for the determination of Nitrosomonas and Nitrobacter have been described previously (6). All inoculated tubes and uninoculated controls were incubated at 28°C for 28 days. Following the incubation period, tubes containing Nitrosomonas broth were examined for ammonium oxidation to nitrite or nitrate by a spot plate test using diphenylamine (7). Nitrobacter medium was tested for nitrite oxidation to nitrate by diphenylamine spot plate test after reduction of residual nitrite by sulfamic acid (7). The tubes were scored positive or negative and the densities of each organism (MPN/100 ml) were determined by using standard MPN tables (8).

Heterotrophic Bacteria

Aerobic heterotrophic bacterial densities both in water and sediments were determined by Spread Plate Technique using Foot and Taylor medium (9) modified by the addition of 100 ppm Actidione (Cycloheximide). The plates were incubated at 20°C for 7 days. Counts were determined with the aid of a Quebec colony counter and are expressed as count per ml.

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Pollution Indicator Bacteria

Water samples only were analyzed for total coliforms, fecal coliforms and fecal streptococci by membrane filtration technique using MOE's Standard Methods (10). The densities of indicator bacteria are expressed as count per 100 ml.

RESULTS

The results of microbiological analyses of water and sediment samples are given in Table 1. A summary of physical and chemical data (provided by the Water Modelling Section) is presented in Table 2. These results are discussed in detail in the following:

Nitrifying Bacteria

The numbers of Nitrosomonas and Nitrobacter were generally low in water and sediment at the upstream station (S5). The densities of these bacteria both in waters and sediments increased downstream of the STP between S6 and S8, but declined slightly at S9 and S10. At the downstream stations (S6-S10), the levels of Nitrosomonas ranged from 460 to 1,100 and 9,300 to 460,000 per 100 ml in waters and sediments, respectively. The populations of Nitrobacter varied from 16 to 1,100 and 9,300 to 75,000 per 100 ml in waters and sediments, respectively, at these stations. With one exception, the maximum concentration of Nitrosomonas and Nitrobacter in both water and sediment were observed at S8. Furthermore, the densities of both nitrifier groups in sediments were significantly greater than in water samples.

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These observations corresponded well with the physical and chemical data (Table 2). For example, the concentrations (mg/l) of free ammonia, Kjeldahl and nitrite nitrogen were relatively low at S5, increased downstream of STP between S6 and S8, and declined to a stable level at S9 and S10. The nitrate concentration also increased downstream of S6, but peaked at S9 and S10, probably as a result of the activity of nitrifiers at S6, S7 and S8.

Likewise, BOD consistently increased between S6 and S8, but declined with the increasing distance from STP. The increased BOD at S6 and S8 was also demonstrated by lower DO values at these stations as compared to S5.

In general, the densities of Nitrosomonas both in waters and sediments were strikingly higher than those of Nitrobacter. These differences in populations may be due to high levels of NH_4^+ (substrate for Nitrosomonas) and relatively low levels of NO_2^- (substrate for Nitrobacter) in the receiving waters. This assumption is supported by the high concentrations of free ammonia and Kjeldahl nitrogen observed, and by low levels of nitrite in the STP effluent (Table 2).

Heterotrophic Bacteria

The concentration of heterotrophic bacteria (indicators of the degree of organic enrichment) ranged from 21,560 to 23,000 per ml in waters. Like the nitrifying bacteria, the levels of heterotrophic bacteria were strikingly higher in sediments than in waters, and varied from 73,000 to 1,730,000 per g (wet wt.) sediment. The maximum heterotrophic density in sediment was observed at S8, and in water at S7.

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Pollution Indicator Bacteria

The densities of total coliform, fecal coliform and fecal streptococcus were generally low in the water at all stations. The lowest levels of these bacteria were detected at S5 and the highest at S6, just below STP. Densities of fecal pollution indicator bacteria declined downstream, with increasing distance from STP.

DISCUSSION AND CONCLUSIONS

Both ammonia- oxidizers (Nitrosomonas) and nitrite- oxidizers (Nitrobacter) were detected in large numbers in Speed River waters and sediments up to approximately 10 kilometers downstream of Guelph STP. Upstream of the STP outfall (S5), these bacteria were present in very low concentrations. In general, maximum densities of these organisms were observed between S6 and S8.

At all stations, the populations of both nitrifying groups in sediments were significantly greater than in the water phase. This corroborates previous observations by Tuffey et al (11), who found higher levels of Nitrobacter and Nitrosomonas in slime and mud than in water in a shallow stream.

As compared to Nitrosomonas, the levels of Nitrobacter were relatively low both in waters and sediments. Similar observations were made by previous investigators (11, 12, 13), who reported higher values of ammonia-oxidizers in diverse environments. The low densities of Nitrobacter may be due to low concentrations of nitrite. In addition, this group tends to be underestimated by the MPN method (12).

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The maximum population of both Nitrosomonas and Nitrobacter in waters and sediments downstream of STP correlates well with the corresponding maximum concentration of organic nitrogen, nitrite and nitrate. The area between S6 and S8 (5 kilometers downstream of STP), where the highest level of Nitrosomonas and lower levels of nitrate were recorded, represents the "Zone of Ammonia Oxidation". Further, the higher levels of Nitrobacter (particularly in sediments) and nitrate between S8 and S10 is indicative of the "Zone of Nitrite Oxidation".

In contrast to the nitrifiers, the levels of fecal pollution indicator bacteria were generally low in waters indicating their effective treatment at STP. Relatively high levels of heterotrophic bacteria, which preferentially utilize carbonaceous compounds, are indicative of organic enrichment. It is probable that heterotrophs also participate in the nitrification process under natural conditions as suggested recently by Isirimah et al (14).

The following conclusions can be drawn from the present study:

1. The presence of nitrifying bacteria in substantial numbers appear to be responsible for observed nitrification in the Speed River downstream of Guelph STP. Further, the greater densities of these organisms in sediments than in the overlying waters point to the fact that the river bed may be the actual site of nitrification.
2. Nitrification seems to be more pronounced between stations S6 and S10 where maximum densities of Nitrosomonas and Nitrobacter were recorded. This section of the Speed River may be identified as the "Zone of Nitrification".
3. The elevated levels of heterotrophic bacteria both in waters and sediments indicate substantial organic enrichment. This is confirmed by the elevated BOD levels.

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4. Since the present microbiological survey was of limited scope, it is difficult to clearly establish that nitrification is occurring in the Speed River near Guelph STP at a level significant enough to be included in Dissolved Oxygen or Water Quality Model. Further intensive microbiological studies will be required to provide such information. The results of this study, however, indicate that the problem of excessive oxygen consumption in the receiving waters may be remedied by including nitrification in sewage treatment facilities.

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FIGURE 1. Location of sampling stations in the Speed River.

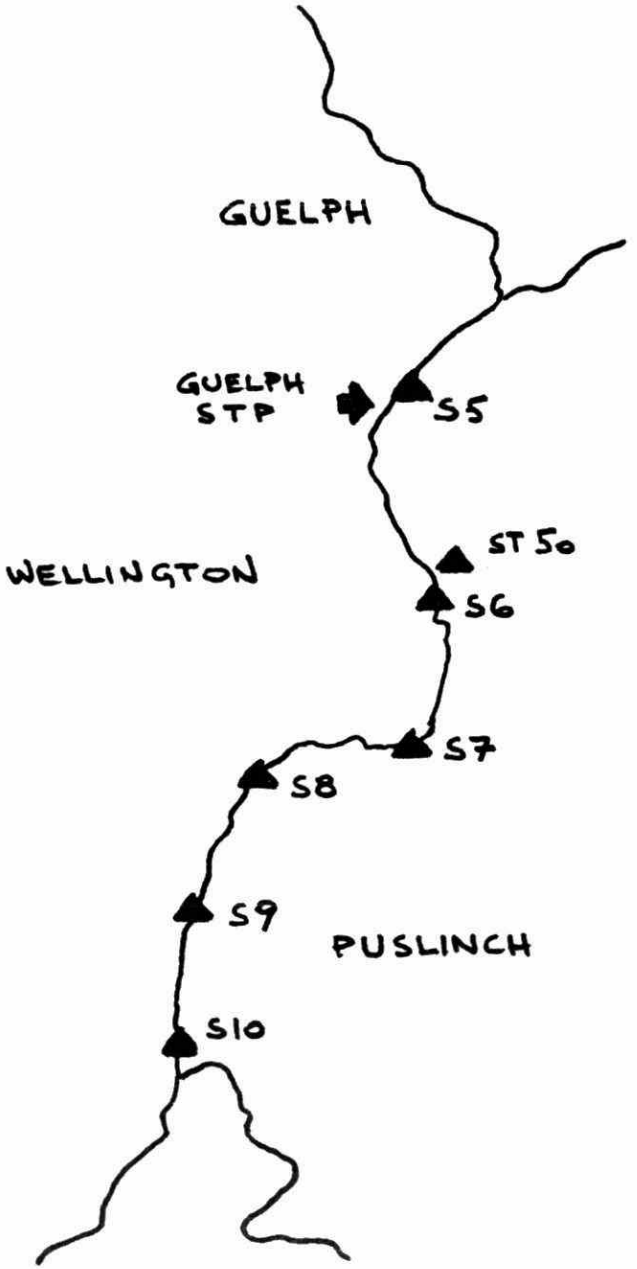

TABLE 1: LEVELS OF POLLUTION INDICATOR, HETEROTROPHIC AND NITRIFYING BACTERIA IN SPEED RIVER WATER AND SEDIMENTS

PHASE	STATION and DISTANCES (Kilometers)	DATE	BACTERIAL PARAMETERS PER 100 ML					
			POLLUTION INDICATOR BACTERIA			NITRIFYING BACTERIA		HETEROTROPHIC BACTERIA per ml
			Total Coliform	Fecal Coliform	Fecal Streptococcus	Nitrosomonas	Nitrobacter	
WATER	S5	8-9-76	800	30	10	20	4	5,930
	(0.00)	9-9-76	300	12	8	240	< 3	4,400
	S6	8-9-76	1,800	256	116	> 1,100	44	6,600
	(2.45)	9-9-76	3,200	156	92	> 1,100	1,100	7,130
	S7	8-9-76	1,500	120	20	> 1,100	> 1,100	7,500
	(4.40)	9-9-76	1,100	56	40	> 1,100	> 1,100	23,000
	S8	8-9-76	300	112	48	> 1,000	460	3,230
	(6.76)	9-9-76	600	148	112	1,100	460	6,160
	S9	8-9-76	1,000	148	76	> 1,100	240	2,560
	(8.69)	9-9-76	900	112	92	1,100	> 1,100	4,260
	S10	8-9-76	600	88	32	460	16	3,230
	(11.12)	9-9-76	500	280	104	1,100	460	5,610

TABLE 1 (cont.) - LEVELS OF POLLUTION INDICATOR, HETEROTROPHIC AND NITRIFYING BACTERIA IN SPEED RIVER WATER AND SEDIMENTS

PHASE	STATION	DATE	BACTERIAL PARAMETERS PER 100 ML					
			POLLUTION INDICATOR BACTERIA			NITRIFYING BACTERIA		HETEROTROPHIC BACTERIA per g[b]
			Total Coliform	Fecal Coliform	Fecal Streptococcus	Nitrosomonas	Nitrobacter	
SEDIMENT	S5	8-9-76	-[a]	-	-	9,300	91	183,000
	S6	8-9-76	-	-	-	93,000	9,300	260,000
	S7	8-9-76	-	-	-	93,000	24,000	520,000
	S8	8-9-76	-	-	-	460,000	46,000	1,730,000
	S9	8-9-76	-	-	-	23,000	24,000	1,160,000
	S10	8-9-76	-	-	-	9,300	75,000	73,000

a = not determined

b = wet weight

TABLE 2. SUMMARY OF PHYSICAL AND CHEMICAL DATA COLLECTED DURING A SIXTY-HOUR SURVEY IN SPEED RIVER.

STATION	TEMP (°C)		D.O (mg/l)		BOD (mg/l)		PHOSPHORUS (mg/l)				NITROGEN (mg/l)							
							TOTAL		SOLUBLE		F. AMMON.		KJELD. N.		NITRITE		NITRATE	
	Mean	Min. Max.	Mean	Min. Max.	Mean	Min. Max.	Mean	Min. Max.	Mean	Min. Max.	Mean	Min. Max.	Mean	Min. Max.	Mean	Min. Max.	Mean	Min. Max.
S5	20.13	17.90	8.36	6.00	1.52	0.80	0.03	0.02	0.00	0.00	0.02	0.00	0.68	0.54	0.00	0.00	0.50	0.46
		23.10		11.20		3.80		0.06		0.00		0.24		1.08		0.01		0.60
S6	19.69	16.75	5.11	3.05	4.68	2.90	0.16	0.08	0.09	0.02	1.32	0.22	2.22	0.94	0.41	0.10	1.39	0.80
		22.95		9.85		6.90		0.26		0.48		2.70		4.59		0.68		2.30
S7	19.86	17.00	4.70	2.20	5.12	3.60	0.15	0.12	0.07	0.03	0.91	0.35	2.00	1.20	0.38	0.30	1.63	1.05
		22.50		8.60		6.20		0.17		0.10		1.50		2.80		0.52		3.22
S8	20.12	18.00	4.72	1.50	3.32	1.40	0.11	0.09	0.05	0.04	0.34	0.01	1.12	0.72	0.21	0.07	2.13	1.10
		22.00		9.10		6.00		0.16		0.07		1.10		1.84		0.36		3.40
S9	19.93	18.00	5.93	2.40	2.72	1.00	0.10	0.07	0.04	0.03	0.17	0.00	0.95	0.66	0.12	0.01	2.39	1.80
		22.10		9.80		6.40		0.14		0.05		0.70		1.66		0.27		3.60
S10	19.92	17.10	6.97	3.90	2.16	0.80	0.09	0.06	0.03	0.03	0.11	0.00	0.87	0.70	0.07	0.01	2.38	1.90
		22.50		10.00		5.00		0.12		0.04		0.50		1.20		0.15		3.50
Guelph STP	19.78	18.90	1.87	1.40	6.53	0.80	1.13	0.85	0.43	0.01	12.26	12.00	17.06	12.60	0.08	0.00	0.50	0.00
		21.00		3.40		24.00		1.60		0.86		18.20		27.00		0.39		1.65

NOTE: All mean values are determined from 15 observations.